

# PATENT ABSTRACTS OF JAPAN

(11)Publication number : 2000-247978

(43)Date of publication of application : 12.09.2000

(51)Int.Cl.

C07D487/22  
A61P 35/00  
A61K 31/4439

(21)Application number : 11-047517

(71)Applicant : TOYO INK MFG CO LTD  
KAWAKAMI HIROYOSHI

(22)Date of filing : 25.02.1999

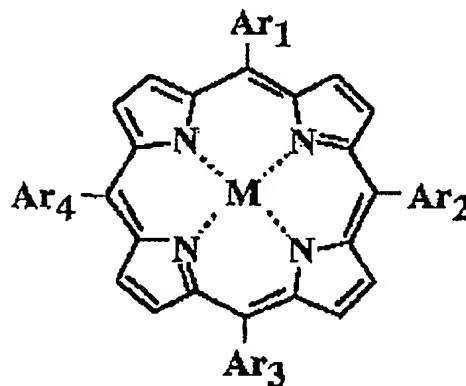
(72)Inventor : KAWAKAMI HIROYOSHI  
NAGAOKA SHOJI  
NAKAMURA KUNIE  
OSE TOSHIYUKI  
MURASE TORU

(54) METAL PORPHYRIN COMPLEX AND PHARMACEUTICAL COMPOSITION CONTAINING THE SAME

(57)Abstract:

**PROBLEM TO BE SOLVED:** To obtain a new compound having high SOD activity and the character to specifically accumulate on a cancer cell and capable of specifically killing cancer cells by inducing hydroxyl freeradicals through reaction with active oxygen, therefore useful as an anticancer agent with slight side effects.

**SOLUTION:** This new compound is a compound of the formula [M is a metal atom for forming a complex; Ar1 to Ar4 are each a (substituted) carbocyclic or heterocyclic aromatic group, at least one of them being a cationic group-bearing aromatic group]. The compound of the formula is obtained, for example, by the following process: pyrrole is reacted with an aromatic aldehyde such as benzaldehyde to form a porphyrin ring moiety, which is then cationized with an alkylating agent (e.g. methyl p-toluenesulfonate) such as a halogenated lower alkyl or lower alkyl tosylate and then converted to the corresponding metal complex using a metal or metal compound (e.g. a metal halide such as iron chloride).



## LEGAL STATUS

[Date of request for examination] 05.09.2005

[Date of sending the examiner's decision of rejection]

[Kind of final disposal of application other than the examiner's decision of rejection or application converted registration]

[Date of final disposal for application]

[Patent number]

[Date of registration]

[Number of appeal against examiner's decision of rejection]

[Date of requesting appeal against examiner's decision of rejection]

[Date of extinction of right]

\* NOTICES \*

JPO and NCIP are not responsible for any damages caused by the use of this translation.

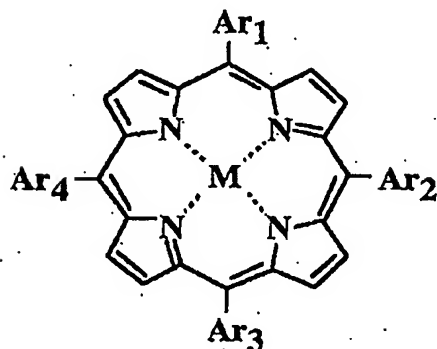
1. This document has been translated by computer. So the translation may not reflect the original precisely.
2. \*\*\* shows the word which can not be translated.
3. In the drawings, any words are not translated.

CLAIMS

[Claim(s)]

[Claim 1] General formula (1)

[Formula 1]



(— M shows the metal atom for forming a complex among a formula, Ar1, Ar2, Ar3, and Ar4 show the ring type which may have a substituent independently, respectively, or a heterocycle type aromatic series radical, and at least one piece is an aromatic series radical which has the cationic radical of Ar1, Ar2, Ar3, and Ar4.) — cationic metalloporphyrin complex expressed.

[Claim 2] The cationic metalloporphyrin complex according to claim 1 whose M is an iron atom, a copper atom, or a manganese atom.

[Claim 3] The cationic metalloporphyrin complex according to claim 1 or 2 whose at least one of Ar1, Ar2, Ar3, and the Ar4 is an N-low-grade alkyl-4-pyridyl radical.

[Claim 4] The cationic metalloporphyrin complex according to claim 3 whose N-low-grade alkyl-4-pyridyl radical is an N-methyl-4-pyridyl radical.

[Claim 5] The cationic metalloporphyrin complex according to claim 1 or 2 whose at least one of Ar1, Ar2, Ar3, and the Ar4 is 4-N, N, and N-Tori low-grade alkylamino phenyl group.

[Claim 6] 4-N, N, and N-Tori low-grade alkylamino phenyl group — 4- the cationic metalloporphyrin complex according to claim 5 which is an N, N, and N-trimethyl aminophenyl radical.

[Claim 7] The physic constituent which comes to contain a cationic metalloporphyrin complex according to claim 1 to 6.

[Claim 8] The HIDOROKI radical induction agent which comes to contain a cationic metalloporphyrin complex according to claim 1 to 6.

[Claim 9] The anticancer agent which comes to contain a cationic metalloporphyrin complex according to claim 1 to 6.

[Claim 10] The physic constituent which comes to contain the hydroxy radical induction agent which can change active oxygen in the living body into a hydroxy radical.

[Claim 11] The physic constituent according to claim 10 whose physic constituent is an anticancer agent.

[Translation done.]

\* NOTICES \*

JPO and NCIP are not responsible for any damages caused by the use of this translation.

1. This document has been translated by computer. So the translation may not reflect the original precisely.
2. \*\*\*\* shows the word which can not be translated.
3. In the drawings, any words are not translated.

DETAILED DESCRIPTION

[Detailed Description of the Invention]

[0001]

[Field of the Invention] This invention relates to a new cationic metalloporphyrin complex and the physic constituent which comes to contain it. In a detail, the physic constituent of this invention is more useful as a SOD activator or an anticancer agent. A cancer cell is easy to be accumulated and the cationic metalloporphyrin complex of this invention is useful as a therapy agent of various alternative neoplasms with few side effects.

[0002]

[Description of the Prior Art] In recent years, many chemical treatment methods by drugs came to be used as the approach of cancer treatment. However, the present condition is that exert an operation also on a normal cell with a cancer cell since many drugs do not have the singularity only to a cancer cell, for this reason an intense side effect cannot be said to be that the lifting and the chemotherapy are not necessarily working effectively. For example, although it is carried out for whether being clinical \*\* that the cisplatin which is one of the anticancer agents used by clinical is effective in a uterine cancer, many side effects are also reported with effectiveness. Moreover, although bioactive protein, such as interferon, TNF, and CSF, attracted attention from having cancer singularity, that the anticancer operation itself is not enough and since it cannot administer orally, it has come to be widely used as an anticancer agent.

[0003] By the way, although the reactive oxygen species induced in the living body are carrying out big work when maintaining the bioactive of a living body, such as annihilating the different-species living thing which invaded in the living body, they are said generation of the active oxygen beyond the need to be not only harmful, but for the reactive oxygen species generated to coincidence in the living body beyond the need to also destroy a self organization, and to be the cause of aging. Although the anticancer agent which annihilates a cancer cell by changing oxygen in the living body into reactive oxygen species, and attacking a cancer cell nonspecific using the toxicity of these reactive oxygen species is also developed, it is nonspecific, and since a normal cell is also attacked, many side effects have been caused.

[0004] Although activating oxygen in the living body, making reactive oxygen species generate, and annihilating a cancer cell, when a certain kind of porphyrin system compound piles up a cancer cell etc. and irradiates laser light on the other hand at this is found out and it has been used as one of the anticancer agents with comparatively high singularity Laser light must be made to irradiate the focus section, and when the focus section is in the interior, it has a fault, such as being ineffective.

[0005]

[Problem(s) to be Solved by the Invention] This invention offers the anticancer agent with few side effects based on the new mechanism of action which acts on a cancer cell specifically. It is reported by the cancer cell that the SOD activity is falling and the burst size of active oxygen is increasing compared with a normal cell (A. V. Peskin, et al., FEBS Lett., 78, 41; (1977) V. Leroy, et al., Cancer Res., 47, and 4771 (1987)). this invention persons thought that the new anticancer mechanism which can attack a cancer cell specifically could be built, when the active oxygen which a cancer cell emits was convertible for the hydroxy radical (-OH) which has higher reactivity on that spot paying attention to this point.

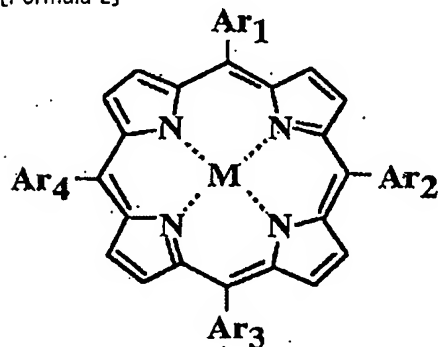
[0006] It found out that this invention persons have the SOD activity excellent in the new metalloporphyrin complex, and have the property specifically accumulated on a cancer cell, could react with active oxygen, and could make a hydroxy radical able to induce, and a cancer cell could be annihilated specifically. That is, this invention offers the new cationic metalloporphyrin complex with which a side effect has little SOD activity with high safety. Moreover, since the new cationic metalloporphyrin complex of this invention accumulates on a cancer cell specifically, it is useful as an anticancer agent with few side effects, and this invention offers a new anticancer agent.

[0007]

[Means for Solving the Problem] This invention is the following general formula (1).

[0008]

[Formula 2]



(-- M shows the metal atom for forming a complex among a formula, Ar1, Ar2, Ar3, and Ar4 show the ring type which may have a substituent independently, respectively, or a heterocycle type aromatic series radical, and at least one piece is an aromatic series radical which has the cationic radical of Ar1, Ar2, Ar3, and Ar4.) -- it is related with the cationic metalloporphyrin complex expressed.

[0009] Moreover, this invention relates to the physic constituent which consists of support permitted on the cationic metalloporphyrin complex expressed with said general formula (1), and medicine manufacture. The physic constituent of this invention is not only a hydroxy radical induction agent, but it can use it as an anticancer agent. Furthermore, this invention relates to the physic constituent which consists of support permitted on the hydroxy radical induction agent which can change active oxygen in the living body into a hydroxy radical on that spot, and medicine manufacture, especially an anticancer agent.

[0010] As for the cancer cell, it is known that antioxidation enzymes (SOD, catalase, etc.) are missing compared with a normal cell, for this reason, as for a cancer cell, the superoxide radical is generated so much compared with a normal cell. For example, if LLC-WRC-256 cell of a cancer cell, the BRL-3 A cell of a normal cell, and the SOD activity of a natural antioxidation enzyme (Cu, Zn-SOD) are measured, it will become as it is shown in the next table 1.

[0011]

[Table 1]

表1 癌細胞と正常細胞におけるSOD活性の比較

細胞	IC <sub>50</sub>
	[×10 <sup>3</sup> U/mg蛋白質]
LLC-WRC-256細胞	8±0.6
BRL-3A細胞	22±1.2
Cu, Zn-SOD	760±45

[0012] Thus, in a cancer cell, SOD activity is decreasing greatly compared with a normal cell. The cationic metalloporphyrin complex expressed with said general formula (1) of this invention has SOD activity, reacts specifically with a superoxide radical in the living body, and generates a hydrogen peroxide. Furthermore, since the cationic metalloporphyrin complex of this invention has the metal at the core, the generated hydrogen peroxide generates a lifting and a toxic high hydroxy radical for a harbor vice mold reaction extremely, and it attacks only a cancer cell specifically.

[0013] On the other hand, in being the normal cell on which the antioxidation enzyme in the living body is functioning, even if a superoxide radical hardly occurs but the cationic metalloporphyrin complex of this invention exists in a normal cell, it cannot react with a superoxide radical and a hydroxy radical cannot be generated. Therefore, alternative and since it is a hydroxy radical induction agent with the outstanding SOD activity, the cationic metalloporphyrin complex of this invention is very useful as a new anticancer agent with high cancer cell singularity with few side effects.

[0014] The cationic metalloporphyrin complex of this invention is characterized by having four aromatic series radicals in a porphin frame, and having the cationic radical of the four aromatic series radicals in at least one piece. even if four aromatic series radicals are the things of a ring type independently, respectively -- the thing of a heterocycle type -- you may be -- that of a monocycle type -- even when -- you may be the thing of a polycyclic type. As an aromatic series radical, it is the radical guided from the benzene ring, a naphthalene ring, etc. as a thing of a ring type, and is the radical guided as a thing of a heterocycle type from the heterocycle of the monocycle type of 5 which has one piece or two nitrogen atoms or more, an oxygen atom, or a sulfur atom -- 10 members, or a condensed-ring type, for example, is the radical guided from a pyridine ring, a pyrimidine ring, an azole ring, etc. A phenyl group, 4-pyridyl radical, etc. are mentioned as a desirable aromatic series radical.

[0015] These aromatic series radicals may have the substituent which does not have a bad influence on SOD activity or an anticancer operation. As a substituent in an aromatic series radical, carbon numbers 1-10, the straight chain of 1-6 or a low-grade branching-like alkyl group, the amino group and the amino group permuted by the above mentioned low-grade alkyl group, the lower alkoxy group that consists of the above mentioned low-grade alkyl group are mentioned preferably.

[0016] As a cationic radical which an aromatic series radical has, although ammonium, a sulfonium radical, etc. are mentioned, quaternary ammonium is desirable. Although you may have the cationic radical of this invention as a substituent of an aromatic series radical, the heteroatom of an aromatic series radical may be cation-ized. as the aromatic series radical which has a cationic radical -- for example, 4- an N, N, and N-trimethyl aminophenyl radical and 4- 4-, such as N, N, and N-triethyl aminophenyl radical, -- N-low-grade alkyl-4-pyridyl radicals, such as N, N, and N-Tori low-grade alkylamino phenyl group, an N-methyl-4-pyridyl radical, and an N-ethyl-4-pyridyl radical, etc. are mentioned.

[0017] Although at least one piece is an aromatic series radical which has the cationic radical of Ar1, Ar2, Ar3, and Ar4 of said general formula (1) of this invention, it has the desirable radical of the cationicity of these aromatic series radicals two or more pieces. As a thing for a porphyrin ring part of a cationic metalloporphyrin complex expressed with said general formula (1) of this invention All of Ar1, Ar2, Ar3, and Ar4 N-low-grade alkyl-4-pyridyl radicals, such as an N-methyl-4-pyridyl radical, or 4- 4-, such as an N, N, and N-trimethyl aminophenyl radical, -- the compound which is N, N, and N-Tori low-grade alkylamino phenyl group -- Ar1, Ar2, and Ar3 N-low-grade alkyl-4-pyridyl radicals, such as an N-methyl-4-pyridyl radical, It is N, N, and N-Tori low-grade alkylamino phenyl group. or 4- 4-, such as an N, N, and N-trimethyl aminophenyl radical, -- The compound whose Ar4 is a phenyl group, and Ar1 and Ar2 N-low-grade alkyl-4-pyridyl radicals, such as an N-methyl-4-pyridyl radical, It is N, N, and N-Tori low-grade alkylamino phenyl group. or 4- 4-, such as an N, N, and N-trimethyl aminophenyl radical, -- The compound Ar3 and whose Ar4 are phenyl groups, and Ar1 and Ar3 N-low-grade alkyl-4-pyridyl radicals, such as an N-methyl-4-pyridyl radical, or 4- 4-, such as an N, N, and N-trimethyl aminophenyl radical, -- the compound Ar2 and whose Ar4 it is N, N, and N-Tori low-grade alkylamino phenyl group, and are phenyl groups is mentioned.

[0018] Although there will be especially no limit as a central metal M in said general formula (1) of this invention if SOD activity and an anticancer operation are shown, an iron atom, a copper atom, or a manganese atom is mentioned preferably.

[0019] The cationic metalloporphyrin complex expressed with said general formula (1) of this invention can be manufactured

according to a well-known approach. For example, a pyrrole and aromatic aldehyde are made to react and a part for the Pori Phi Lynne ring part is manufactured, and this can be cation-ized with alkylating agents, such as halogenation low-grade alkyl and low-grade alkyl tosylate, and it can manufacture by the approach of subsequently using as a metal complex using a metal or metallic compounds, for example, a metal halogenide etc.

[0020] this invention persons considered the anticancer operation of various kinds of metalloporphyrin complexes using several sorts of cancer cells from which LLC-WRC and 256 cell of the walker rat (Walker rat) cancer origin and SOD activity differ as a cancer cell. The procedure of this trial is typically shown in drawing 1. In addition, the compound which examined, and its abbreviated name are as follows.

[0021] FeTM4PyP: The iron complex whose Ar1, Ar2, Ar3, and Ar4 of a general formula (1) are an N-methyl-4-pyridyl radical.

MnTM4PyP: The manganese complex whose Ar1, Ar2, Ar3, and Ar4 of a general formula (1) are an N-methyl-4-pyridyl radical.

CuTM4PyP: The copper complex whose Ar1, Ar2, Ar3, and Ar4 of a general formula (1) are an N-methyl-4-pyridyl radical.

FeTMAP: iron complex whose Ar1, Ar2, Ar3, and Ar4 of a general formula (1) are a 4-N, N, and N-trimethyl aminophenyl radical.

FeTSPP: iron complex whose Ar1, Ar2, Ar3, and Ar4 of a general formula (1) are 4-sulfonate phenyl group.

[0022] Preparation of a porphyrin solution was performed using culture medium, and it adjusted so that porphyrin concentration might finally become [ ml ] in 10 or 50,100microg /. Measurement added and cultivated the metalloporphyrin complex solution to the cancer cell developed on 12 hole plate, three days after addition, measured the rate of cell survival of each cancer cell by the trypan blue staining technique, and evaluated the anticancer effectiveness.

[0023] Moreover, it is the SOD activity of these metalloporphyrin complexes T. Ohse, et al., Porphyrins, 6, and 137 (1997) According to the approach indicated, the disproportionation rate constant (kcat) of the active oxygen (O2-) by the stopped-flow method estimated. kcat made the HEPES/HEPES-Na buffer solution (pH8.1) of the Pori Phi Lynne complex, and the DMSO solution of KO2 react at 36 degrees C, and was calculated from attenuation with an absorbance of 245nm which is the absorption maximum wavelength of O2-. kcat and IC50 in a metalloporphyrin complex and an antioxidation enzyme are shown in the next table 2. In addition, SOD activity is so high that the value of IC50 \*\* is so small that the value of kcat is large.

[0024]

[Table 2]

表2 各種ポルフィリン錯体のkcat値とIC50

試験化合物	kcat [ $\times 10^{-4} \text{M}^{-1} \text{s}^{-1}$ ]	IC50 [ $\mu\text{g/ml}$ ]
Cu, Zn-SOD	2310	0.3
FeTM4PyP	22	0.8
FeTM4PyMPP	5.4	1.6
FeDM4PyDPP	3.8	1.8
MnTM4PyP	22	0.7
FeTSPP	---	---

[0025] Furthermore, the intracellular SOD activity which a cancer cell has prepared the homogenate solution of a cell, and computed it with the chemiluminescence method using CLA. And the generated radical kind (-OH) was measured by ESR which used DMPO as a spin trap agent.

[0026] The rate of extinction of the cancer cell using the cationic metalloporphyrin complex (100microg/(ml)) of invention by LLC-WRC and 256 cell and the antioxidation enzyme (50microg/(ml)) of the bovine red cell origin is shown in drawing 2. As a result of the rate evaluation of cell survival by the trypan blue staining technique of LLC-WRC and 256 cell, an antioxidation enzyme has the anticancer effectiveness very low [ activity ] in itself, although it has SOD activity. Moreover, by metaled comparison, FeTM4PyP with high SOD activity and -OH production ability showed the highest anticancer effectiveness. It is thought that Mn complex which has the outstanding SOD activity depends on low -OH production ability [ that effectiveness was seldom accepted ] of Mn complex. With Cu complex in which \*\*\*\*\* and OH production ability are shown like Fe complex on the other hand, since SOD activity is low, it is thought that the anticancer effectiveness became low compared with Fe complex.

[0027] Moreover, the aforementioned result is shown in drawing 3 with the value of the disproportionation rate constant (kcat) of the active oxygen (O2-) used as the index of SOD activity. The numeric value in drawing 3 was 106xkcat (M-1s-1), and, incidentally the value of an antioxidation enzyme was 2300x106 (M-1s-1).

[0028] Next, this invention persons examined the accumulation ability to the cancer cell of a cationic metalloporphyrin complex. In order to consider the compatibility with a cell membrane and permeability it is opaque from a lipid membrane double layer, it examined about the following compound with which a hydrophilic property differs from hydrophobicity.

[0029] FeTM4PyP: The iron complex whose Ar1, Ar2, Ar3, and Ar4 of a general formula (1) are an N-methyl-4-pyridyl radical.

FeTM4PyMPPP: The iron complex whose Ar4 Ar1, Ar2, and Ar3 of a general formula (1) are an N-methyl-4-pyridyl radical, and is a phenyl group.

Fecis-DM4PyDPP: The iron complex Ar3 and whose Ar4 Ar1 and Ar2 of a general formula (1) are an N-methyl-4-pyridyl radical, and are phenyl groups.

Fetrans-DM4PyDPP: The iron complex Ar2 and whose Ar4 Ar1 and Ar3 of a general formula (1) are an N-methyl-4-pyridyl radical, and are phenyl groups.

[0030] The accumulation behavior to a cancer cell was checked by observing the red fluorescence of a porphyrin complex using a fluorescence microscope. More furthermore than atomic absorption analysis, the quantum of the intracellular porphyrin complex was carried out. A result is shown in the next table 3.

[0031]

[Table 3]

表3 各種ポルフィリン錯体の癌細胞への集積能

試験化合物	取り込み量 [ $\text{fmol}/\text{細胞}$ ]
FeTM4PyP	$1.4 \pm 0.1$
FeTM4PyMPP	$1.9 \pm 0.2$
FeDM4PyDPP	$11 \pm 0.8$
MnTM4PyP	$1.4 \pm 0.2$
FeTSP	$1.0 \pm 0.1$

[0032] Consequently, it was checked that a hydrophobic, stronger porphyrin complex has higher accumulation ability. Especially as for FeDM4PyDPP, many complexes were incorporated by the cancer cell in the inside of a short time. By introducing a hydrophobic radical into a porphyrin complex, an interaction with a canal-cell membrane becomes strong and it is thought that accumulation ability increased.

[0033] Moreover, a change of the anticancer effectiveness about these compounds (100microg/(ml)) with time was measured. This result is shown in drawing 4. In the black trigonum mark in drawing 4, the black dot mark shows FeTM4PyMPP and the black square mark shows FeDM4PyDPP for FeTM4PyP, respectively. The used cancer cell is LLC-WRC and 256 cell of the walker rat (Walker rat) cancer origin, and each compound was used for it by 100microg [ / ml ] concentration. The axis of ordinate of drawing 4 shows the survival rate of a cancer cell, and the condition that all cancer cells survive is shown 100%.

[0034] FeDM4PyDPP which has two phenyl groups in a meso position annihilated almost all cancer cells within [ in 24 hours ] after addition. The anticancer effectiveness decreased in order of FeDM4PyDPP > FeTM4PyP > FeTM4PyMPP.

[0035] The result of having measured the value of fifty percent lethal dose (Median Lethal Dose: amount of drugs required to annihilate a cell 50%) is shown in Table 4. The value of kcat about the SOD activity measured by the approach collectively mentioned above is shown in Table 4.

[0036]

[Table 4]

表4 各種ポルフィリン錯体のkcat値とLD50

試験化合物	kcat [ $\times 10^{-4} \text{M}^{-1} \text{s}^{-1}$ ]	LD50 [ $\mu\text{M}/\text{ml}$ ]
FeTM4PyP	22	46
FeTM4PyMPP	5.4	150
FeDM4PyDPP	3.8	24
FeTM4PyP	22	--
FeTSP	--	--

[0037] The disproportionation rate constant (kcat) fell with reduction of a cationic substituent, and became the order of FeTM4PyP > FeTM4PyMPP > FeDM4PyDPP. However, FeDM4PyDPP made the anticancer operation in which has SOD activity and high accumulation ability was excellent induce, though it is low. That is, when accumulation to a cancer cell increases remarkably by the hydrophobicity of FeDM4PyDPP, it is thought that the fall of the SOD activity by reduction of a cationic substituent was compensated, and the outstanding anticancer operation was shown. In the anticancer effectiveness that a cationic metalloporphyrin complex shows the accumulation ability from the above thing to a cancer cell, it turned out that it is an important factor.

[0038] Next, the operation over the normal cell of the compound of this invention was considered. First, the growth experiment of a normal cell (BRL-3A) was conducted using the mitomycin-C which is the compound and the well-known anticancer antibiotic of this invention. A result is shown in drawing 5. The black dot mark in drawing 5 shows control, the black square mark shows the case where FeTM4PyP is added, the black trigonum mark shows the case where MnTM4PyP is added, and a white round mark shows the case where mitomycin-C is added. When the mitomycin-C which is the conventional anticancer agent is added, the number of cells decreases with the passage of time, and, about 70 hours after, it is set to 0. On the other hand, in the case of the compound of this invention, growth of a cell as well as [ almost ] control is performed. That is, it turns out that the compound of this invention hardly affects a normal cell.

[0039] Furthermore, FeTM4PyP which is the compound of this invention was added by various concentration in the culture medium of a normal cell (BRL-3 A cell) and a cancer cell (Walker256 cell), and the survival rate of each cell was measured. A result is shown in drawing 6. The white round mark in drawing 6 shows the survival rate of a cancer cell, and the black dot mark shows a normal cell. In a cancer cell, even if the concentration of FeTM4PyP raises concentration in the normal cell to the survival rate falling to about 50%, and concentration falling [ g / // 100micro ] to about 40% by ml further by ml in 50microg / , it turns out that the survival rate hardly falls and the compound of this invention hardly affects it to a normal cell.

[0040] From the above result, when the active principle of this invention generated a hydroxy radical specifically in the cancer cell which has much active oxygen, it became clear that it was what does the anticancer effectiveness so. The anticancer agent by such mechanism provides the cancer cell by the mechanism with new this invention with a specific new anticancer agent based on the new idea by this invention persons. Moreover, the physic constituent of this invention prescribes [ taking orally or ] for the patient continuously whether generally 1micro g-1g is prescribed for the patient in several steps per day, although a medicine can be prescribed more for the patient parenteral and the effective dose is different with symptoms and a patient. The physic constituent of this invention can be pharmaceutical-preparation-ized by the well-known approach, and can be suitably

manufactured by the medication method or the patient.

[0041]

[Example] Next, although this invention is concretely explained based on an example, this invention is not limited to these examples.

[0042] Example 1 (composition of the Pori Flynn ring)

300ml of propionic acids beforehand dehydrated by Na<sub>2</sub>SO<sub>4</sub> was put into the three neck flask, and under nitrogen, at 110 degrees C, benzaldehyde 7.5ml (0.0707 is l) and pyridine-4-aldehyde 12.5ml (0.1165 mols) were put in, and it agitated and shaded. Subsequently, pyrrole 12.5ml (0.1863 mols) was dropped slowly. The solvent was distilled off after making it react for 2 hours.

Aqueous ammonia neutralized and the solvent was distilled off. Reduced pressure drying was performed at 100 degrees C. Washing filtration was carried out with the methanol and reduced pressure drying of the residue was carried out. Separation purification of the porphyrin (TPP, MPyTPP, DPyDPP, TPyMPP, TPyP) was carried out in the flash plate column (stationary phase: silica gel, 95% of expansion solvent:methylene chloride 100%→ methanol 5% / methylene chlorides). The cis object of DPyDPP and the trans object were inseparable this time. Isolation of each porphyrin was checked by NMR. Yield was about 2%.

[0043] Example 2 (methylation of a porphyrin (class[ the / fourth ]-izing of N atom of the pyridyl radical of a meso position)) The solvent (ethanol 10% (3ml) / chloroform 90% (27ml)) was put into the three-neck flask, and each porphyrin (in the case [ In the case / In the case of MPyTPP / of 0.077g (1.3x10<sup>-4</sup>Mol) and DPyDPP ] of 0.235g (3.8x10<sup>-4</sup> mol) and TPyMPP 0.251g (3.9x10<sup>-4</sup> to 4 mol)) was added. A number of N atom of p-toluenesulfonic-acid methyls (to MPyTPP, it is 0.88ml (4.7x10<sup>-4</sup> to 3 mol) to 0.57ml (3.1x10<sup>-4</sup> to 3 mol) and TPyMPP to 0.120ml (6.4x10<sup>-4</sup> to 4 mol) and DPyDPP) of about 4 time molar quantity to methylate were added, and it shaded under 35 degrees C and nitrogen, and was made to react all night. After distilling off a solvent, reduced pressure drying was performed and MMPyTPP (what methylated MPyTPP), DPyDPP (what methylated DPyPPP), and TMPyMPP (what methylated TPyMPP) were obtained.

[0044] TMPyMPP was melted to the methanol of \*\*\*\* after this, was reprecipitated in diethylether, and collected the residue after filtration. Since the DMPyPPP was very more refractory, this process was not performed. Reduced pressure drying was fully performed. Moreover, since MMPyTPP was insoluble in water, it was not able to be used here. The check of each methylation was performed by NMR (refer to drawing 7, drawing 8, and drawing 9). Yield was about 45%.

[0045]

1 H-NMR [270MHz, DMSO-d<sub>6</sub>]: delta DMPyDPP 9.48 (8H, 2, 6-pyridyl)

9.20 (8H, pyrrole - beta)

9.00 (8H, 3, 5-pyridyl)

4.73 (12H, N-methyl)

- 3.10 (2H, internal pyrrole)

TPMyMPP 9.71-7.30(46H)

4.96 (9H, N-methyl)

- 2.74 (2H, internal pyrrole)

TPMyP 9.44-7.07(40H)

4.71 (6H, N-methyl)

- 2.90 (2H, internal pyrrole)

[0046] Example 3 (metal (Fe) installation to a porphyrin)

150ml (pH4.05) of succinic-acid buffer solutions is put into a three neck flask. Under 80 degrees C and nitrogen Each porphyrin (in DMPyDPP, 0.257g (2.7x10<sup>-4</sup> to 4 mol)) In TMPyMPP, 0.207g (1.8x10<sup>-4</sup> to 4 mol) is added. It is the about 10 time molar quantity (as opposed to DMPyDPP 0.425g (2.1x10<sup>-4</sup> to 3 mol)) of a porphyrin about ferric chloride (FeCl<sub>3</sub>) 4 hydrate. 0.382g (1.9x10<sup>-4</sup> to 3 mol) is put in to TMPyMPP, and a UV-Vis spectrum is measured for every hour, and it was made to react until it was able to check metal installation from the shift of a peak, and an absorbance. The iron which separated in the column (stationary phase: 10% of expansion solvent:water → water, ion-exchange-resin HP20 and methanol 90%) was separated after distilling off a solvent, reduced pressure drying after distilling off was performed for the solvent, and FeDMPyDPP and FeTMPyMPP were obtained. Yield was about 70%.

[0047] Example 4 (cell culture)

After thawing the cell which carried out cryopreservation with 37-degree C warm water, except for DMSO which moves to a centrifugation tube, carries out centrifugal separation by 1000 rotations for 10 minutes, throws away a supernatant, and is used in the case of cryopreservation, culture medium (it expresses MEM hereafter.) was put in, and after suspension, it moved to the culture flask and cultivated within the incubator (under 37 degrees C and carbon dioxide gas). After it exchanged MEM every two to three days and a cell fully increased, trypsinization was carried out, the cell was stripped from the flask, and it moved to the centrifugation tube, and for 10 minutes, the after [ centrifugal separation ] supernatant was thrown away by 1000 rotations, MEM was added, it moved to some flasks after suspension (passage), and the cell was proliferated. Under the present circumstances, LLC-WRC-256 cell is Eagle's. essential medium (it expresses E-MEM hereafter.) and BRL-3 A cell are Ham's. It cultivated using F-12K (it expresses F-12K hereafter.).

[0048] Example 5 (carcinostatic activity evaluation)

The cell which carried out subculture was stripped from the culture flask by trypsinization, and it moved to the centrifugation tube, and for 10 minutes, after centrifugal separation and a supernatant were thrown away by 1000 rotations, MEM was added, and every 1ml per hole was added to the dish of 12 holes after suspension. Since it will cultivate by the incubator for one day and the cell was implanted, each porphyrin complex prepared so that final concentration might become in ml and ten to 100 microg /and mitomycin-C, or carboplatin was added, and it put into the incubator. Evaluation was taken out from the incubator after fixed time amount progress (0.5 - 120 hours after), and it carried out by dyeing an extinction cell with a trypan blue solution. MEM was attracted after fixed time amount progress, the trypan blue solution was put in after washing by PBS, and it put into the incubator for 15 minutes. It washed after [ PBS ] suction, PBS was put in again, and a photograph was taken by observing under a culture microscope (OLYMPUS handstand mold culture microscope IMT-2) (OLYMPUS microphotography automatic exposure photography equipment modelPM-10-A). The number of the extinction cell dyed by the trypan blue from the acquired photograph and the viable cells which have not dyed was measured, and the rate of cell survival was computed. The above procedure is typically shown in drawing 1.

[0049]

[Effect of the Invention] This invention offers the therapy approach of cancer by the new mechanism of annihilating a cancer cell specifically, and the new anticancer agent by this approach, by changing into a hydroxy radical ( $-OH$ ) the active oxygen which exists in a cancer cell so much. The anticancer agent of this invention has few side effects over a normal cell, and its curative effect is safely high. Moreover, this invention offers the new metalloporphyrin complex which has growth control and an anticancer operation of a cancer cell.

[Translation done.]



**\* NOTICES \***

JPO and NCIP are not responsible for any damages caused by the use of this translation.

1. This document has been translated by computer. So the translation may not reflect the original precisely.
2. \*\*\*\* shows the word which can not be translated.
3. In the drawings, any words are not translated.

---

**DESCRIPTION OF DRAWINGS**

**[Brief Description of the Drawings]**

[Drawing 1] Drawing 1 shows a measuring method for the survival rate of a cancer cell typically.

[Drawing 2] Drawing 2 graph-izes the rate of extinction to the cancer cell of the cationic metalloporphyrin complex of this invention, and an antioxidation enzyme, and shows it.

[Drawing 3] Drawing 3 graph-izes the rate of extinction to the cancer cell of various metalloporphyrin complexes and an antioxidation enzyme, and shows the kcat value of the SOD activity.

[Drawing 4] Drawing 4 graph-izes the survival rate over the cancer cell of the cationic metalloporphyrin complex of this invention, and shows it. In the black trigonum mark in drawing 4, the black dot mark shows FeTM4PyMPP and the black square mark shows FeDM4PyDPP for FeTM4PyP, respectively.

[Drawing 5] Drawing 5 graph-izes the operation over the normal cell in the cationic metalloporphyrin complex and the well-known anticancer agent of this invention, and shows it.

[Drawing 6] Drawing 6 graph-izes effect of the cancer cell and normal cell by the cationic metalloporphyrin complex on this invention, and shows it.

[Drawing 7] Drawing 7 shows the NMR chart of the compound before metalization of the cationic metalloporphyrin complex (DMPyDPP) of this invention.

[Drawing 8] Drawing 8 shows the NMR chart of the compound before metalization of the cationic metalloporphyrin complex (TMPyMPP) of this invention.

[Drawing 9] Drawing 9 shows the NMR chart of the compound before metalization of the cationic metalloporphyrin complex (TMPyP) of this invention.

---

**[Translation done.]**

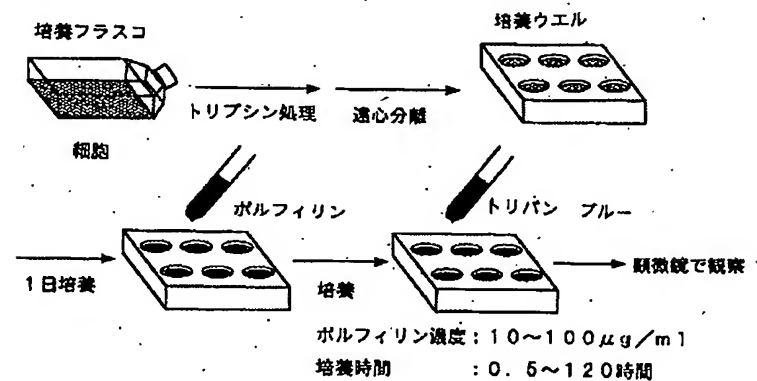
\* NOTICES \*

JPO and NCIP are not responsible for any damages caused by the use of this translation.

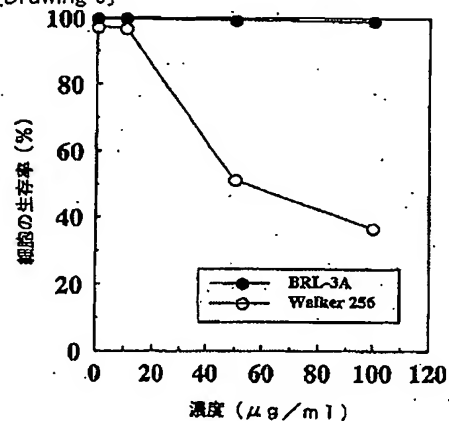
1. This document has been translated by computer. So the translation may not reflect the original precisely.
2. \*\*\*\* shows the word which can not be translated.
3. In the drawings, any words are not translated.

DRAWINGS

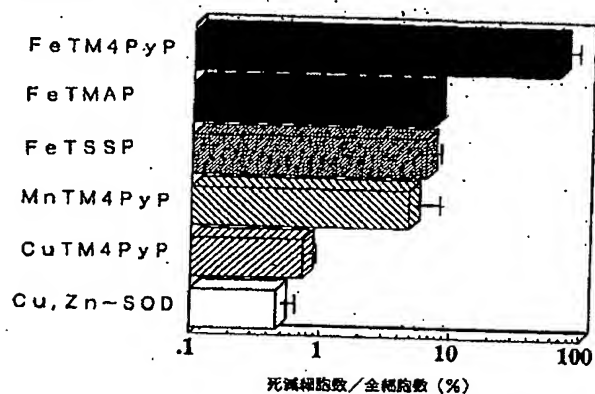
[Drawing 1]



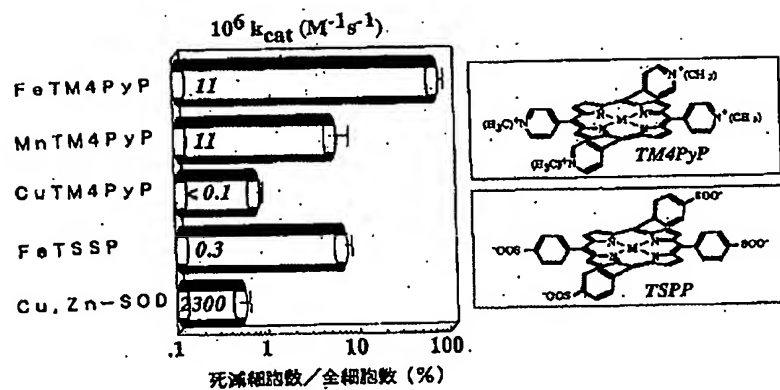
[Drawing 6]



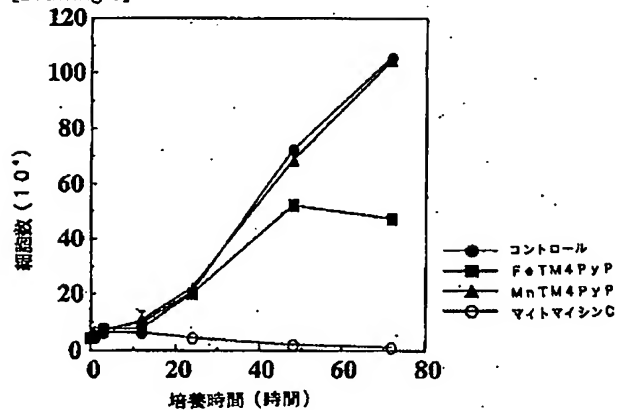
[Drawing 2]



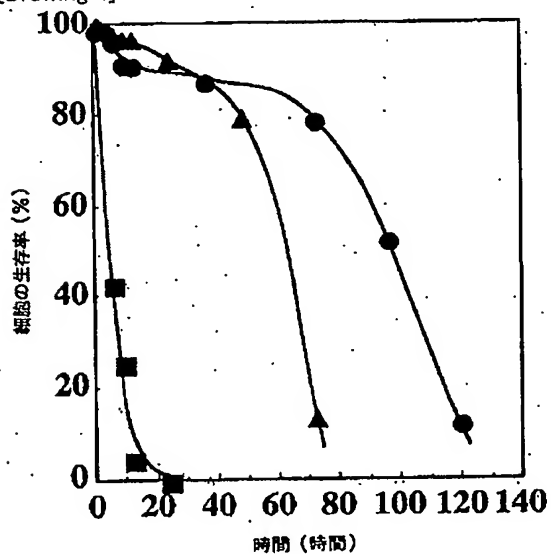
[Drawing 3]



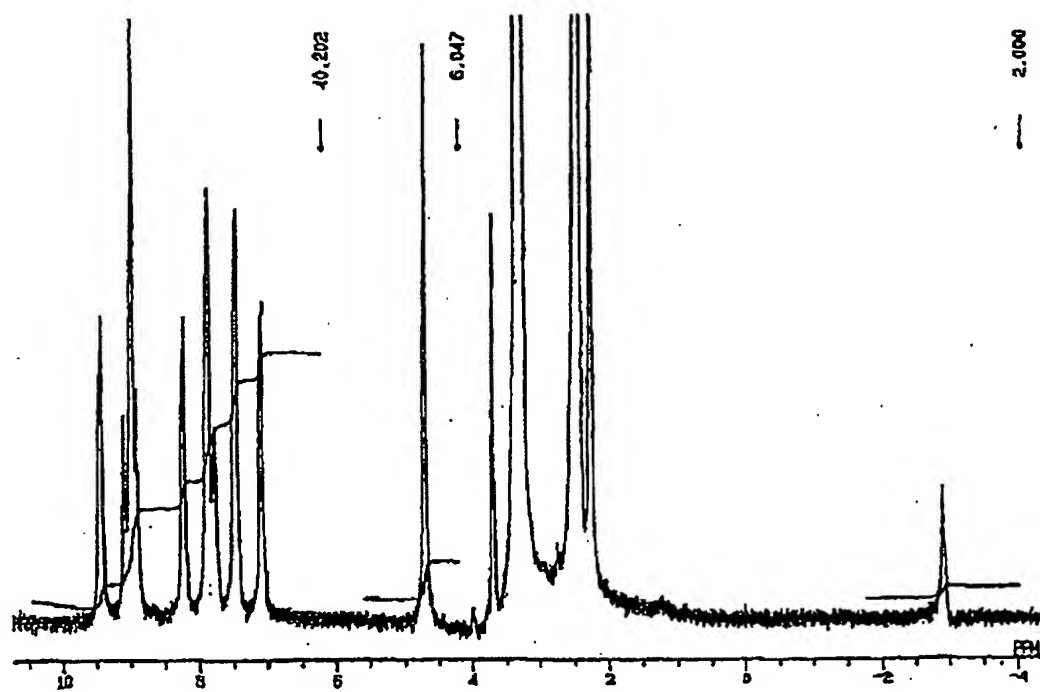
[Drawing 5]



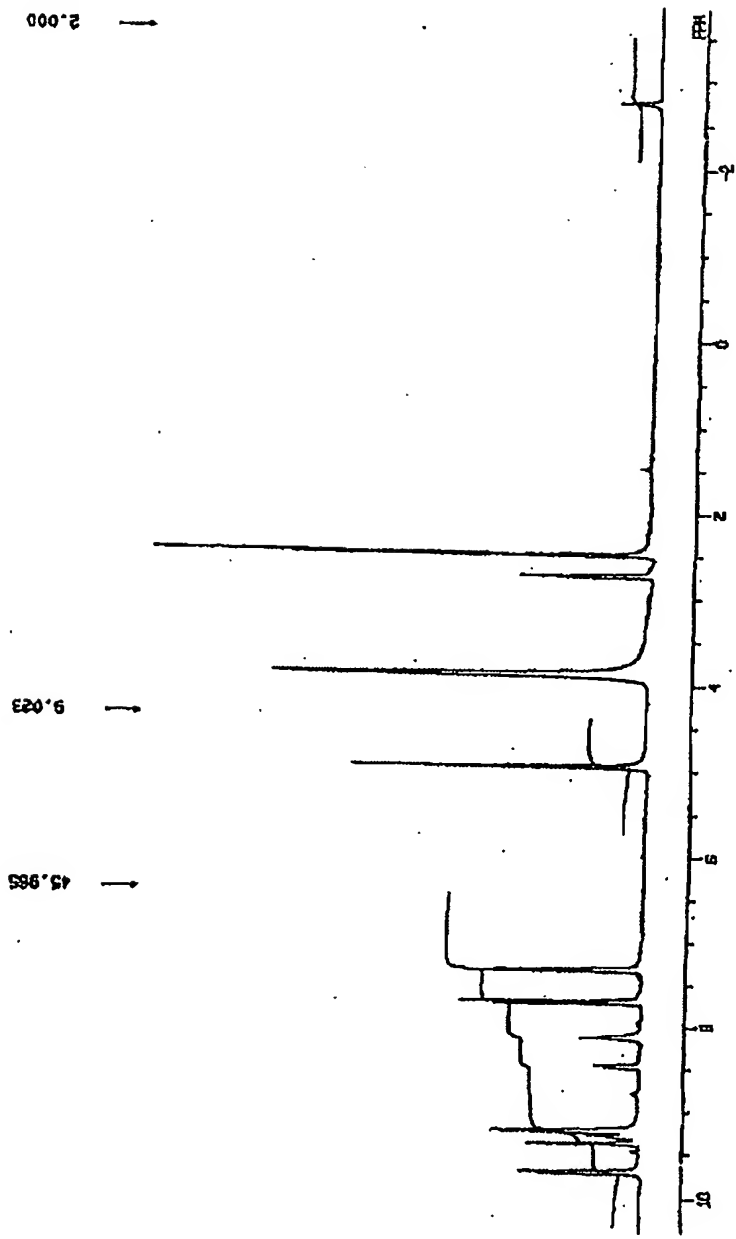
[Drawing 4]



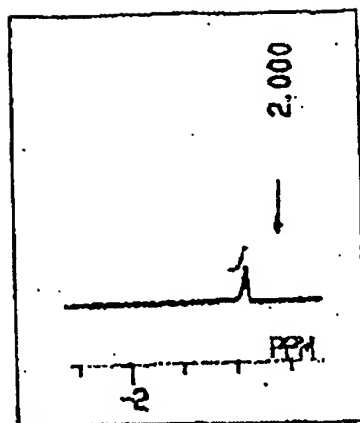
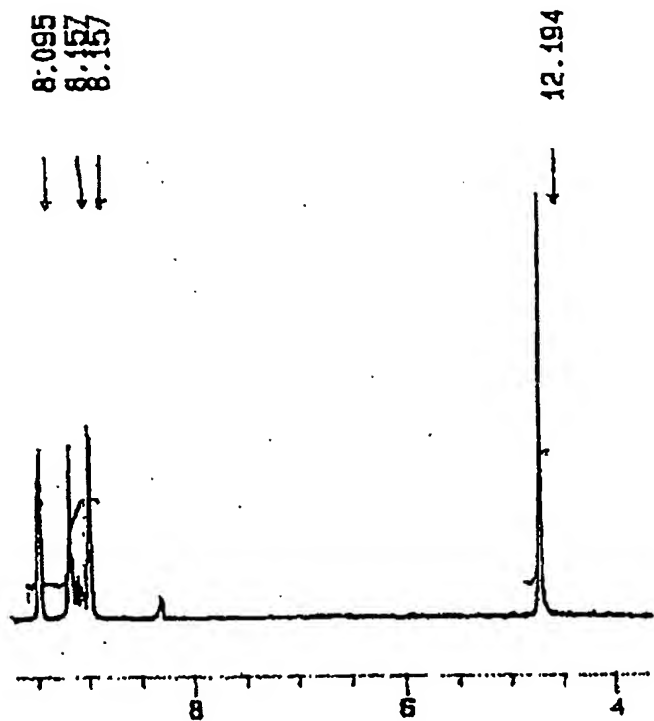
[Drawing 7]



[Drawing 8]



[Drawing 9]



[Translation done.]